

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
Human caspase-5 (SEQ ID NO:)
Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
Human caspase-5 (SEQ ID NO:)
Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
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conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
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Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

FIGURE 1

FIGURE 2

	1				50
hCaspase-12	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Ap	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Bp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Cp	MADEKPSXGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Dp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Jp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
KW-Kp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLNTDE	IHLIGKCLKF
	51				100
hCaspase-12	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
KW-Ap	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Bp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Cp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Dp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~PSGKL
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Jp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Kp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
	101				150
hCaspase-12	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-ApIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-BpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-CpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-DpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ep	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Fp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Gp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Hp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ip	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-JpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Kp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
	151				200
hCaspase-12	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Ap	LDLLGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Bp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Cp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Dp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Ep	LDLLGM*DLL	ENLGYSVVIK	ENLTA....
KW-Fp	LDLLGM*DLL	ENLGYSVVIK	ESLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Gp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Hp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQ....
KW-Ip	LDLLGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLA
KW-Jp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQEME.STFLV
KW-Kp	LDLLGM*DLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV

	201				250
hCaspase-12	FMSHGILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
KW-Ap	FMSHSILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
KW-Bp	FMSHSILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
KW-Cp
KW-Dp
KW-Ep	...SILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
KW-Fp	FMSHSILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
KW-Gp
KW-Hp
KW-Ip	FMSHSILNRI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPK.....
KW-Jp	FMSHSILNGI	CGTKH~~~~	~~~~~	~~~~~	~~~~~
KW-Kp	FMSHSILNGI	CGTKHWDQEP	DVLHDDTIFE	IFNNRNCQSL	KDKPKVIIMQ
	251				300
hCaspase-12	ACRGNAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-Ap	ACRGNAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-Bp	ACRGNAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-CpGAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-DpGAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-Ep	ACRG~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Fp	AC~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-GpGAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-HpGAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
KW-IpGAGIV	WFTTDVEKAS	ADTHGRLLQG	NICNDAVTKV	HVEKDFIAFK
KW-Jp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Kp	ACRGNAGIV	WFTTDSGKAS	ADTHGRLLQG	NICNDAVTKA	HVEKDFIAFK
	301				350
hCaspase-12	SSTPHNVSWR	HETNGSVFIS	QIIYYFREYS	WSHHLEEIFQ	KVQHSFETPN
KW-Ap	SSTPHNVSWR	HETNGSVFIS	QIIYYFREYS	WSHHLEEIFQ	KVQHSFETPN
KW-Bp	SSTP.....VQHSFETPN
KW-Cp	SSTPHNVSWR	HETNGSVFIS	QIIYYFREYS	WSHHLEEIFQ	KVQHSFETPN
KW-Dp	SSTP.....VQHSFETPN
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Gp	SSTP.....VQHSFETPN
KW-Hp	SSTPHNVSWR	HETNGSVFIS	QIIYYFREYS	WSHHLEEIFQ	KVQHSFETPN
KW-Ip	SSTP.....VQHSFETPN
KW-Jp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Kp	SSTPHNVSWR	HETNGSVFIS	QIIYYFREYS	WSHHLEEIFQ	KVQHSFETPN
	351		374		
hCaspase-12	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Ap	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Bp	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Cp	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Dp	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Ep	~~~~~	~~~~~	~~~~~		
KW-Fp	~~~~~	~~~~~	~~~~~		
KW-Gp	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Hp	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Ip	ILTQLPTIER	LSMTRYFYLF	PGN*		
KW-Jp	~~~~~	~~~~~	~~~~~		
KW-Kp	ILTQLPTIER	LSMTRYFYLF	PGN*		

L_a_aln

Human Caspase-12 compared to Mouse Caspase-12 with CARD domain, ICE-p20 domain, ICE-p10 domain and Active-site amino acids described.

hCaspase-12	<u>MADEKPSNGVLVHMKLLIKTFLDGIFFDLMENNVLNTDEIHLIGKCLKFVVSNAENLVD</u>	60
mCaspase-12	<u>MAARRTHERDPIYKIKGLAKOMLDGVFDDLVEKNVLNGDELLKIGESASFILNKAENLVE</u>	60
	** . . . : : : * * * : * * * : * * * : * * * : * * * : * * * : * * * :	
hCaspase-12	<u>DITETAQIAGKIFREHLWNSKKOLS</u> -----	85
mCaspase-12	<u>NFLEKTDMAKGI FAGHIANSOEQLSLOFSNDEDDGPQKICTPSSPSESKRKVEDDEMEVN</u>	120
	:: * . . . : * * * : * * * : * * * : * * * : * * * : * * * : * * * :	
	↑auto catalytic	
hCaspase-12	----- <u>SALLEIQGAQPSGKLKLCPHAHFHELKTKRADEIYPVMEKERRTCLALN</u>	134
mCaspase-12	<u>AGLAHESHLMLTAPHGLQSSEVQDTLKLCPRDQFCKIKTERAKEIYPVMEKEGRTLALI</u>	180
	: * : * . . . : * * * : * * * : * * * : * * * : * * * : * * * :	
	↑calpain	
hCaspase-12	<u>IRNKEFNYLHNRNGSELDLLGMRDLENLGYSVVIKENLTAQEMETALROFAAHPEHQSS</u>	194
mCaspase-12	<u>ICNKKFDYLFDRDNADTDILNMQELLENLGYSVVLKENLTAQEMETELMQFAGRPEHQSS</u>	240
	* * * : * * * : * * * : * * * : * * * : * * * : * * * : * * * : * * * :	
hCaspase-12	<u>DSTFLVFMSHGILNGICGTXHWDQEPDVLHDDTIFEIFNNRNCOSLKDCKPKVIIMQACRG</u>	254
mCaspase-12	<u>DSTFLVFMSHGILEGICGVKXRNKKPDVLHDDTIFKIFNNSNCRSLRNKPKILIMQACRG</u>	300
	***** : * * * : * * * : * * * : * * * : * * * : * * * : * * * :	
hCaspase-12	<u>NGAGIVWFTTDSGKASADTHGRLLQGNICNDAVTKAHVEKDFIAFKSSTPHNVSWRHETN</u>	314
mCaspase-12	<u>RYNGTIWVSTNKGIAATADTDEERVLSCWNNSTKXHVETDFIAFKSSTPHNISWKVGKT</u>	360
	. * : * . . . : * * * : * * * : * * * : * * * : * * * : * * * :	
	↑auto catalytic	
hCaspase-12	<u>GSVFISQIIYYFREYSWSHLEEIFOKVOHSFETPNILTQPTIERLSMTRYFYLFPGN</u>	373
mCaspase-12	<u>GSLFISKLIDCFKKYCWCYHLEEIFRKVOHSFEVPGELTQPTIERVSMTRYFYLFPGN</u>	419
	** : * * * : * * * : * * * : * * * : * * * : * * * : * * * : * * * :	

Legend for Domains as calculated by PFAM

CARD Domain xxxxxxx
ICE-p20 Domain yyyyyyy
ICE-p10 Domain zzzzzzz

Active-Site Residues: h...c

Calpain and Auto-catalytic cleavage sites determined for Mouse Caspase-12

FIGURE 3

H12_a_~1

CLUSTAL W (1.7) multiple sequence alignment

```

h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 -----MADE
m_Caspase-12 -----MAAR
h_Caspase-4 -----MAEG
h_Caspase-13 -----MAED
h_Caspase-5 -----MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKD
h_Caspase-1 -----MAD
h_Caspase-6 -----
h_Caspase-8 -----MDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPKIDALM
h_Caspase-10 MKSQGQHWYSSSDKNCKVSVREKLLIIDSNLGVQDVENLKFLCIGLVPNKKLEKSSSSASD
h_Caspase-9 -----MDE
h_Caspase-2 -----MAADRGRIRILGVCGM
h_Caspase-14 -----

h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 KPSNGVLVHMVK---LLIKTFLDGI--FDDLMEENVLNTDEIHLIGKCL-KFVVSNAEN
m_Caspase-12 RTERDPIYKIK---GLAKDMLDGV--FDDLVEKNVLNGDELLKIGESA-SFILNKAEN
h_Caspase-4 N-HRKKPLKVLE---SLGKDFLTGV--LDNLVEQNVNLNWKEEEKKKYYD-AKTEDKVRV
h_Caspase-13 K-HNKNPLKMLE---SLGKELISGL--LDDFVEKNVLKLEEEKKKKYYD-AKLQDKARV
h_Caspase-5 N-HKKKTVMKLE---YLGKDVHGV--FNYLAKHDVLTLEEEKKKKYYD-AKIEDKALI
h_Caspase-1 KVLKEKRKLFIR---SMGEGTINGL--LDELLQTRVLNKEEMEKVKREN-ATVMDKTRA
h_Caspase-6 -----
h_Caspase-8 LFQRLQEKRMLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRV
h_Caspase-10 VFEHLLAEDLLSEEDPFFLAELLYIIR-QKKLLQHLNCTKEEVERLLPTR--QRVSLFRN
h_Caspase-9 ADDRLLRRCLR-----LVEELQVDQLWDALLSSELFPHMIEDIQRAGSGSRRDQARQ
h_Caspase-2 HPHHQETLKKNR---VVLAKQLLLSELLEHLLEKDIITLEMRELIQAKV--GSFSQNV
h_Caspase-14 -----

h_Caspase-3 -----MENTEN-----SVDSK-SIKNLEPKIIH---GSE-----
h_Caspase-7 ---MADDQGCIEEQGVEDSANED---SVDAPDRSSFVPSLFS---K-KKKN-----
h_Caspase-12 LVDDITETAQIAGKIFREHLWNS---KKQLSSALL--EIQGAQ---PSG---K---
m_Caspase-12 LVENFLEKTD MAGKIFAGHIANS---QEQLSLQFSNDEDDGPQKICTPSSPSES KRKV
h_Caspase-4 MADSMQEKQRMAGQMLLTFFNID---QISPNKKAHPNMEAGPP---ESGES-----
h_Caspase-13 LVDSIRQKNQEAGQVFVQTFLNID---KNSTSIKAPETVAGPD---ESVGS-----
h_Caspase-5 LVDSLRL-KNRVAHQMFQTQTLNMD---QKITSVKPLLQIEAGPP---ESAES-----
h_Caspase-1 LIDSVIPKGAQACQICITYICEEDS---YLAGTLGLSADQTS GNYLNMQDSQGVLS SFFPA
h_Caspase-6 -----MSSASGLRRGHAPAGGE-----EN-----
h_Caspase-8 MLYQISEEVSRSELRSFKFLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRV
h_Caspase-10 LLYELSEGIDSENKDMIFLLKDSL P-KTEM TSLSFLAFLEKQGK---IDEDNLTCLEDL
h_Caspase-9 LIIDLETRGSQALPLFISCLEDTG---QDMLASFLRTNRQA AKLSKPTLENLTPVVL RP
h_Caspase-2 LLNLLPKRGPQAFDAFCEALRETKQGHLEDMLLTTL SGLQHVLPPLSCDYDL SL PFPVCE
h_Caspase-14 -----

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FIGURE 4

h_Caspase-3	-----SMDS--GISLDN-----
h_Caspase-7	-----VTMRS--IKTTRDRVPTY----
h_Caspase-12	----- LKLCPHAHFHELKTKRADE ----
m_Caspase-12	EDDEMEVNAGLAHES--HLM---LTAPHGLQSSEVQDTLKLCPRDQFCKIKTERAKE---
h_Caspase-4	-----TDALKLCPHEEFRLRCKERAEE----
h_Caspase-13	-----AATLKLCPHEEFRLRCKERAGE----
h_Caspase-5	-----TNILKLCPREEFLRLCKKNHDE----
h_Caspase-1	PQAVQD-----NPAMPTSSGSEGNVKLCSLEEAQRIWKQKSAE----
h_Caspase-6	-----MTETDAFYKREMFDPAE----
h_Caspase-8	CAQINKSLLKIINDY--EEFSKERSSSLEGSPDEFSNGEELCGVMTISDSPREQDSE---
h_Caspase-10	CKTVVPKLLRNIEKYKREKAIQIVTPPVDKEAESYQGEELVSQTDVKTFLALPQESWQ
h_Caspase-9	EIRK-----PEVLRPETPRPVDIGSGGFGDVGALSLRGAD---
h_Caspase-2	SCPLYKKLR-----LSTDTVEHSLDNKDGPCVCLQVKPCTPEFYQTHFQ----
h_Caspase-14	-----

h_Caspase-3	-----SYKMDYPEMGLCIIINNKN
h_Caspase-7	-----QYNMNFELGKCIINNKN
h_Caspase-12	----- IYPVMEKERRTCLALNIRN
m_Caspase-12	-----IYPVMEKEGRTRLALIICN
h_Caspase-4	-----IYPIKERNNRTRLALIICN
h_Caspase-13	-----IYPIKERKDRTRLALIICN
h_Caspase-5	-----IYPIKKREDRRRLALIICN
h_Caspase-1	-----IYPIMDKSSRTRLALIICN
h_Caspase-6	-----KYKMDHRRRGIALIFNHER
h_Caspase-8	-----SQTLDKVYQMKSKPRGYCLIINNHN
h_Caspase-10	NKHAGSNGNRATNGAPSLVSRGMQASANTLNSETSTKRAAVYRMNRNHRGLCVIVNNHS
h_Caspase-9	-----LAYILSMEPGCHCLIINNHN
h_Caspase-2	-----LAYRLQSRPRGLALVLSNVH
h_Caspase-14	-----MSNPRSLEEEKYDMSGARLA

:

h_Caspase-3	FH-----KSTGMTSRSGTDVDAANLRETFRNLYEVRNK-NDLTREEIIVELMRDVSKE
h_Caspase-7	FD-----KVTGMGVRNGTDKDAEALFKCFRSLGFDVIVY-NDCSCAKMQDLLKKASEE
h_Caspase-12	K-----EFNYLHNRNGSELDLLGMRDLLENLGYSVVIKENLTAQEMETALRQFAAHP
m_Caspase-12	K-----KFDYLFDRDNADTDILNMQELLENLGYSVVLKENLTAQEMETELMQFAGRP
h_Caspase-4	T-----EFDHLPPrNGADFDITGMKELLEGLDYSVDVEENLTARDMESALRAFATR
h_Caspase-13	T-----EFDHMPPrNGAALDILGMKQLLEGLGYTVEVEEKLTAARDMESVLWKFAARE
h_Caspase-5	T-----KFDHLPARNGAHYDIVGMKRLQLGLGYTVVDEKNLTARDMESVLRAFAARP
h_Caspase-1	E-----EFDSIPRRTGAEVDITGMTMLLQNLGYSVDVKKNLTAASDMTTELEAFARHP
h_Caspase-6	FF-----WHLTLPERRRTCADRDNLTTRFSDLGFEVKCFNDLKAEEILLKIHEVSTVS
h_Caspase-8	FAKAREKVPKLHSIRDRNGTHLDAGALTTTFFELHFEIKPH-DDCTVEQIYEILKIYQLM
h_Caspase-10	F-----TSLKDRQGTHKDAEILSHVFQWLGFVTHIHNNTKMEMVLQKQKCNP
h_Caspase-9	FCR-----E-SGLRTRTGSNIDCEKLRRRFSSPHFMVEVKGDLTAKKMVLALLELAQOD
h_Caspase-2	FTG-----EKELEFRSGGDVDHSTLVTLFKLLGYDVHVLCDQTAQEMQEKLNFAQLP
h_Caspase-14	L-----ILCVTKAREGSEEDLDALEHMFQRFRFESTMKRDPATAEQFQEELEKFOQAI

* * : : : :

h_Caspase-3
h_Caspase-7
h_Caspase-12
m_Caspase-12
h_Caspase-4
h_Caspase-13
h_Caspase-5
h_Caspase-1
h_Caspase-6
h_Caspase-8
h_Caspase-10
h_Caspase-9
h_Caspase-2
h_Caspase-14

↓
D--HSKRSSFVVCVLLSHGEEG-----IIFGTNG-----PVDLKKITNFFRGDRCRSL
D--HTNAACFACILLSHGEEN-----VIYKDG-----VTPIKDLTAHFRGDRCKTL
E--HQSSDSTFLVFMSHGILN-----GICGTHWDQEPDVLHDDTIFEIFNNRNCQSL
E--HQSSDSTFLVFMSHGILE-----GICGVKHRNKKPDVLHDDTIFKIFNNSNCRSL
E--HKSSDSTFLVLMMSGHILE-----GICGTVHDEKKPDVLLYDTIFQIFNNRNCCLSL
E--HKSSDSTFLVFMSHGILD-----GICGTMHSEEPDVLPHYDTIFRTFNNRNCLSL
E--HKSSDSTFLVLMMSGHILE-----GICGTAHKKKKPDVLLYDTIFQIFNNRNCCLSL
E--HKTSDSTFLVFMSHGIRE-----GICGKKHSEQVPDILQLNAIFNMLNKNCPSL
---HADADCFVCVFLSHGEGN-----HIYAYDA-----KIEIQTLTGLFKGDKCHSL
D--HSNMDCFICCILSHGDKG-----IIYGTG-----QEAPIYELTSQFTGLKCPSL
A--HADGDCVFCILTHGRFG-----AVYSSDE-----ALIPIREIMSHFTALQCPRL
---HGALDCCVVVILSHGCQASHLQFPQAVYGTG-----CPVSVEKIVNIFNGTSCPSL
A--HRVTDSCIVALLSHGVEG-----AIYGVG-----KLLQLQEVFQLFDNANCPSL
DSREDPVSCAFVVLMAHGREG-----FLKGEDG-----EMVKLENLFEALNNKNQCAL

::: **

h_Caspase-3
h_Caspase-7
h_Caspase-12
m_Caspase-12
h_Caspase-4
h_Caspase-13
h_Caspase-5
h_Caspase-1
h_Caspase-6
h_Caspase-8
h_Caspase-10
h_Caspase-9
h_Caspase-2
h_Caspase-14

↓
TGKPKLFI IQACRG--ELDCGIETDSG-----VDDDMAC-----HKIP
LEKPKLFFI IQACRG--ELDDGIQADSG-----PINDTDANPR-----YKIP
KDKPKVIMQACRGN--GAGIVWFTTD-----SGKASADTHG-RLLQGNIC--NDAVTKA
RNKPKILIMQACRGR--YNGTIWVSTN-----KGIATADTDEERVLS---CKWNN SITKA
KDKPKVII IQACRGA--NRGELWVRDSP-----ASLEVASSQSSENLE-----EDAVYKT
KDKPKVII IQACRGA--NRGELWVSDSP-----PALADSFQSSENLE-----EDAVYKT
KDKPKVII IQACRGE--KHGELWVRDSP-----ASLAVISSQSSENLE-----ADSVCKI
VKGPKIFII IQACRGN--SPGVVWFKDSV-----GVSGNLSLPTTEEFE-----DDAIKKA
AGKPKVFFI IQACQGDNYQKGIPVETDS-----EEQPYLEMDLS-----SPQTRYI
AEKPKLFFI IQACQGEI QPSVSI EADALN--PEQAPTSLQ-----DSI
GGKPKLFFI IQACGGEQKDHGFEVASTSPEDESPGSNPEPDATPFQEGRLTFDQLDAISSL
QNKPKMFFI IQACRGDETDRGVDQDQDGN-----HAGSPGCEESDAGKE-----KLPKMRL
RAKPKVYII IQACRGEQRDPGETVGGDE-----IVMVIKDSP-----QTI

***: : : ** *

h_Caspase-3
h_Caspase-7
h_Caspase-12
m_Caspase-12
h_Caspase-4
h_Caspase-13
h_Caspase-5
h_Caspase-1
h_Caspase-6
h_Caspase-8
h_Caspase-10
h_Caspase-9
h_Caspase-2
h_Caspase-14

VDA-DFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYA-DKLEFMHILTRVNRKVATE
VEA-DFLFAYSTVPGYYSWRSPGRGSWFVQALCSILEEHG-KDLEIMQILTRVNDRVARH
HVEKDFIAFKSSTPHNVSWRHETNGSVFISQIIYYFREYS-WSHLEEIFQ----KVQHS
HVETDFIAFKSSTPHNISWKVGKTGSLFISKLIDCFKKYC-WCYHLEEIFR----KVQHS
HVEKDFIAFCSSTPHNVSWRDSTMGSIFITQLITCFQKYS-WCCHLEEVFR----KVQQS
HVEKDFIAFCSSTPHNVSWRDIKKGSLFITRLITCFQKYA-WCCHLEEVFR----KVQQS
HEEKDFIAFCSSTPHNVSWRDRTRGSIFITELITCFQKYS-CCCHLMEIFR----KVQKS
HIEKDFIAFCSSTPDNVSWRHPTMGSVFIGRLIEHMQEYA-CSCDVEEIFR----KVRFS
PAGADFLMCYSVAEGYYSHRETVNGSWYIQDLCEMLGKYG-SSLEFTELLTLVNRKVSQR
PDEADFLLGMA TVNVCVSYRNP AEGTWYIQSLCQSLRERCPRGDDILTILT---EVN YE
PAEADFLGLATVPGYVSFRHVEEGSWYIQSLCNHLKKLVPRMLKFLEKTM---EIRGR
PTPSDIFVSYSTFPGFVSWRDPKSGSWYVETLDDIFEQWA-HSEDLQSLLL---RVANA
PTRSDMICGYACLKGTAAAMRNTRKGSWYIEALAQVFSERA-CDMHVADMLVKVN-ALIKD
PTYTDALHVYSTVEGYIAYRHDQKGSCFIQTLVDVFTKRK---GHILELLT---EVTRR

* : : : * : : : : : : : : : : :


```

h_Caspase-3      FESFSFDATFHAKKQIPCIVSMLTKE--LYFYH---
h_Caspase-7      FESQSDDPHFHEKKQIPCVVSMLTKE--LYFSQ---
h_Caspase-12     FET----PNILTQLPTIERLSMTRYF--YLFPGN---
m_Caspase-12     FEV----PGELTQMPTIERVSMTRYF--YLFPGN---
h_Caspase-4      FET----PRAKAQMPTIERLSMTRYF--YLFPGN---
h_Caspase-13     FEK----PNVKAQMPTVERLSMTRYF--YLFPGN---
h_Caspase-5      FEV----PQAKAQMPTIERATLTRDF--YLFPGN---
h_Caspase-1      FEQ----PDGRAQMPTTERTVTLTRCF--YLFPGH---
h_Caspase-6      RVD FCKDPSAIGKKQVPCFASMLTKK--LHFFPKSN-
h_Caspase-8      VSN--KDDKKNMGKQMPQPTFTLRKK--LVFPSD---
h_Caspase-10     KRTVWG-AKQISATSLPTAISAQTPRPPMRRWSSVS-
h_Caspase-9      VSV-----KGIYKQMPGCFNLRKK--LFFKTS--
h_Caspase-2      REGYAPGTEFHRCKEMSEYQSTLCRH-LYLFPGHPPT
h_Caspase-14     MAEAELVQEGKARKTNPEIQSTLTKR--LYLQ-----

```

Legend:

- ↓ Active-site Residues
- * Identical Residues
- : Conservative Substitution
- . Allowable Substitution

H12_b_~1

CLUSTAL W (1.7) multiple sequence alignment

```

h_Caspase-4 -----MAEGN-HRKKPLKVLES
h_Caspase-5 MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKDN-HKKKTVMKLEYL
h_Caspase-13 -----MAEDK-HNKNPLKMLES
h_Caspase-12 -----MADEKPSNGVLVHMKLL
h_Caspase-1 -----MADKVLKEKRKLFIRSM
: : : : :

h_Caspase-4 GKDFLTGVLDNLVEQNVLNWKEEEKKKYYDAKTEDKVRVMADSMQEKQRMAGQMLLQTF
h_Caspase-5 GKDVHGVFNYLAKHDVLTLEEEKKKKYYDAKIEDKALILVDSL-KNRVAHQMFQTLL
h_Caspase-13 GKELISGLLDDFVEKNVLKLEEEKKKKYYDAKLQDKARVLVDSIROKNQEAGQVFVQTF
h_Caspase-12 IKTFLDGIFDDLMENNVLNTDEIHLIGKCLKFVVSNAENLVDDITETAQIAGKIFREHLW
h_Caspase-1 GEGTINGLLDELLQTRVLNKEEMKVKRENATVMDKTRALIDSVIPKGAQACQICITYIC
: : *::: : : **..* . . . : *: : . * : : :

h_Caspase-4 N-----IDQISPNKKAHPNMEAG--PPESGESTDALKLCP
h_Caspase-5 N-----MDQKITSVKPLLQIEAG--PPESAESTNLIKLC
h_Caspase-13 N-----IDKNSTSIKAPETVAG--PDESVGSAATLKLCP
h_Caspase-12 N-----SKKQLSS--ALLEIQGA--QP-SGK----LKLCP
h_Caspase-1 EEDSYLAGTLGLSADQTSGNLYLNMQDSQGVLSFPAPQAVQDNPAMPTSSSGSEGNVKLCS
: : . . . * :***.

h_Caspase-4 HEEFLRLCKERAEIYPIKERNNRTRLALII CNTEFDHLP PRNGADFDITGMKELLEGLD
h_Caspase-5 REEFLRLCKKNHDEIYPIKKREDRRRLALII CNTKFDHLP ARNGAHYDIVGMKRLLQGLG
h_Caspase-13 HEEFLKLCKERAGEIYPIKERKDRTRLALII CNTEFDHMP PRNGAALDILGMKQLLEGLG
h_Caspase-12 HAHFHELKTKRADEIYPVMEKERRTCLALNIRNKEFNYLHNRNGSELDLLGMRDLENLG
h_Caspase-1 LEEAQRIWKQKSAEIYPIMDKSSRTRLALIICNEEFDSIPRRTGAEVDITGMTMLLQNLG
. :. :. ****: .: * *** * * :*: : *:*: *: ** *:*.

h_Caspase-4 YSVDVEENLTARDMESALRAFATRPEHKSSDSTFLVLM SHGILEGICGTVHDEKKPDVLL
h_Caspase-5 YTVVDEKNLTARDMESVLRAFAARPEHKSSDSTFLVLM SHGILEGICGTAHKKKKPDVLL
h_Caspase-13 YTVEVEEKL TARDMESVLWKFAAREEHKSSDSTFLVFM SHGILDGICGTMHSEEPDVLP
h_Caspase-12 YSVVIKENLTAQEMETALROFAAHPEHQSSDSTFLVFM SHGILNGICGTKHWDQEPDV LH
h_Caspase-1 YSVDVKKNLTASDMTTELEAFARPEHKTS DSTFLVFM SHGIREGICGK KHSEQVPDILQ
*: * :*:*** :* : * ** : *:***:*****:***** :****. * : :*: *

h_Caspase-4 YDTIFQIFNNRNCLSLKDKPKVII VQACRGANRGE LWVR-DSPASLEVASSQSSE-NLEE
h_Caspase-5 YDTIFQIFNNRNCLSLKDKPKVII VQACRG EKHGELWVR-DSPASLAVISSQSSE-NLEA
h_Caspase-13 YDTIFRTFNRRNCLSLKDKPKVII VQACRGANRGE LWVS-DSPPALADSFSQSSE-NLEE
h_Caspase-12 DDTIFEI FNNRNCQSLKDKPKVIIMQACRGNGAGI VWFTTDSGKASADTHGRLLQGNICN
h_Caspase-1 LNAIFNMLNTKNCPSLKDKPKVII I QACRGDSPGVVWFK-DSVGVSGNLSLPTTE-EFED
: :*. :*: :*: * *****:***** * :. ** : : :

h_Caspase-4 DAVYKTHVEKDFIAFCSSTPHNVSWRDSTMG SIFITQLITCFQKYSWCCHLEEVFRKVQ
h_Caspase-5 DSVCKIHEEKDFIAFCSSTPHNVSWRDRTRG SIFITELITCFQKYS CCCHLMEIFRKVQ
h_Caspase-13 DAVYKTHVEKDFIAFCSSTPHNVSWRDIKKGSLFITRLITCFQKYAWCCHLEEVFRKVQ
h_Caspase-12 DAVTKAHVEKDFIAFKSSTPHNVSWRHETNGSVFISQIIYYFREYSWSHHLEEFQKVQH
h_Caspase-1 DAIKKAHIEKDFIAFCSSTPDNVSWRHPTMG SVFIGRLIEHMQEYACSCDVEEIFRKVRF
*: : * * ***** ***** . **:* :* :*: :. : :*:*:

h_Caspase-4 SFETPRAKAQMPTIERLSMTRYFYLFPGN
h_Caspase-5 SFEVPQAKAQMPTIERATLTRDFYLFPGN
h_Caspase-13 SFEKPNVKAQMPTVERLSMTRYFYLFPGN
h_Caspase-12 SFETPNILTQLPTIERLSMTRYFYLFPGN
h_Caspase-1 SFEQPDGRAQMPTTERTVLTTRCFYLFPGH
*** * :*:** ** :*: *****:

```

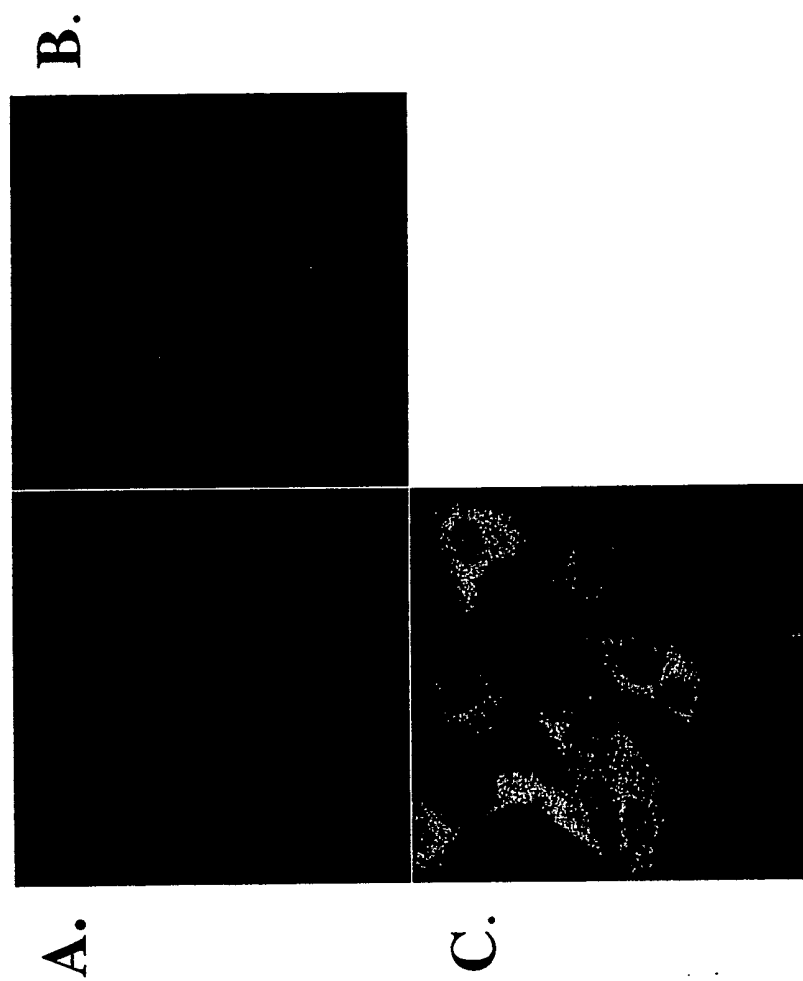



FIGURE 7

SH-EP cell transfection +/- α -Fas

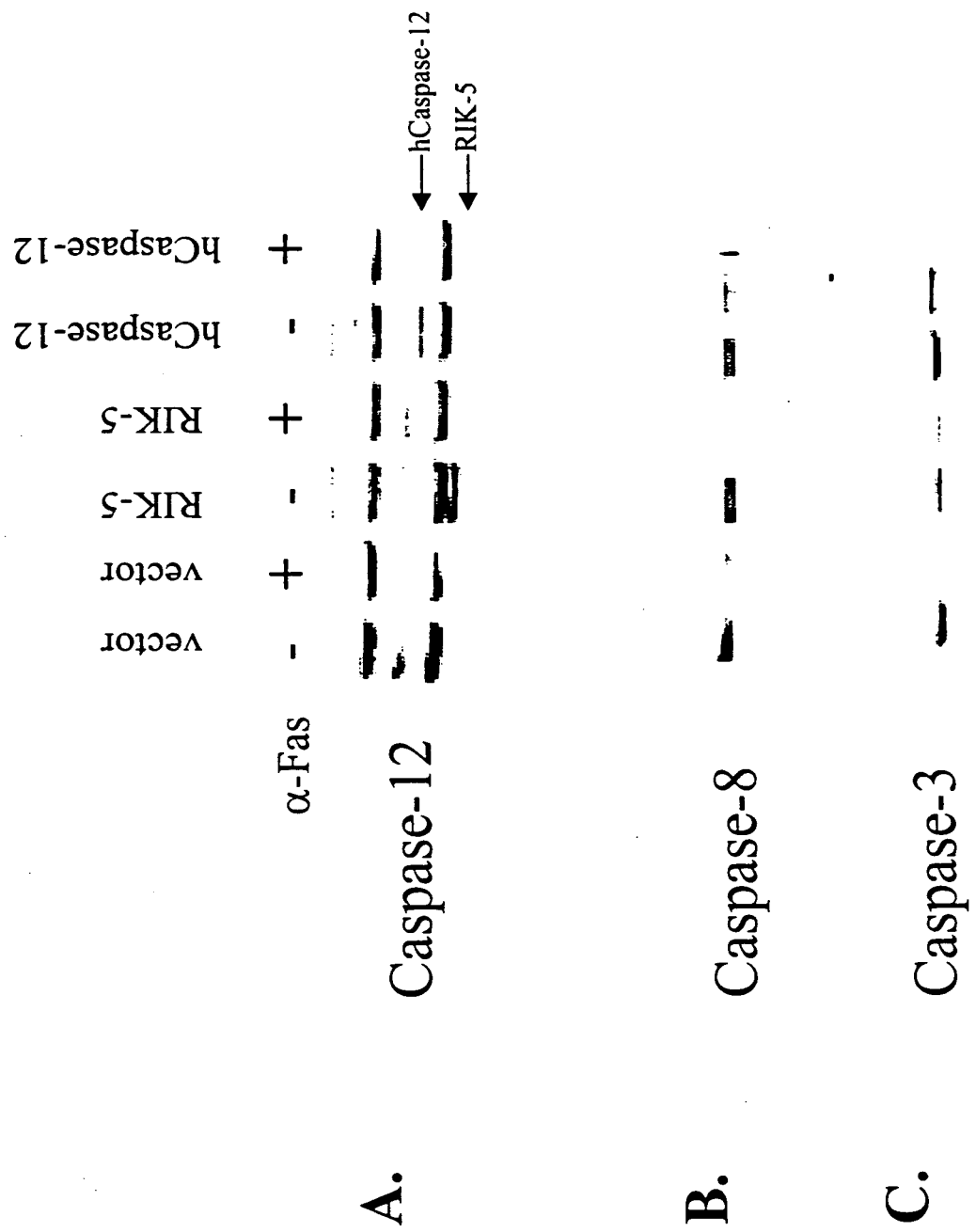


FIGURE 9

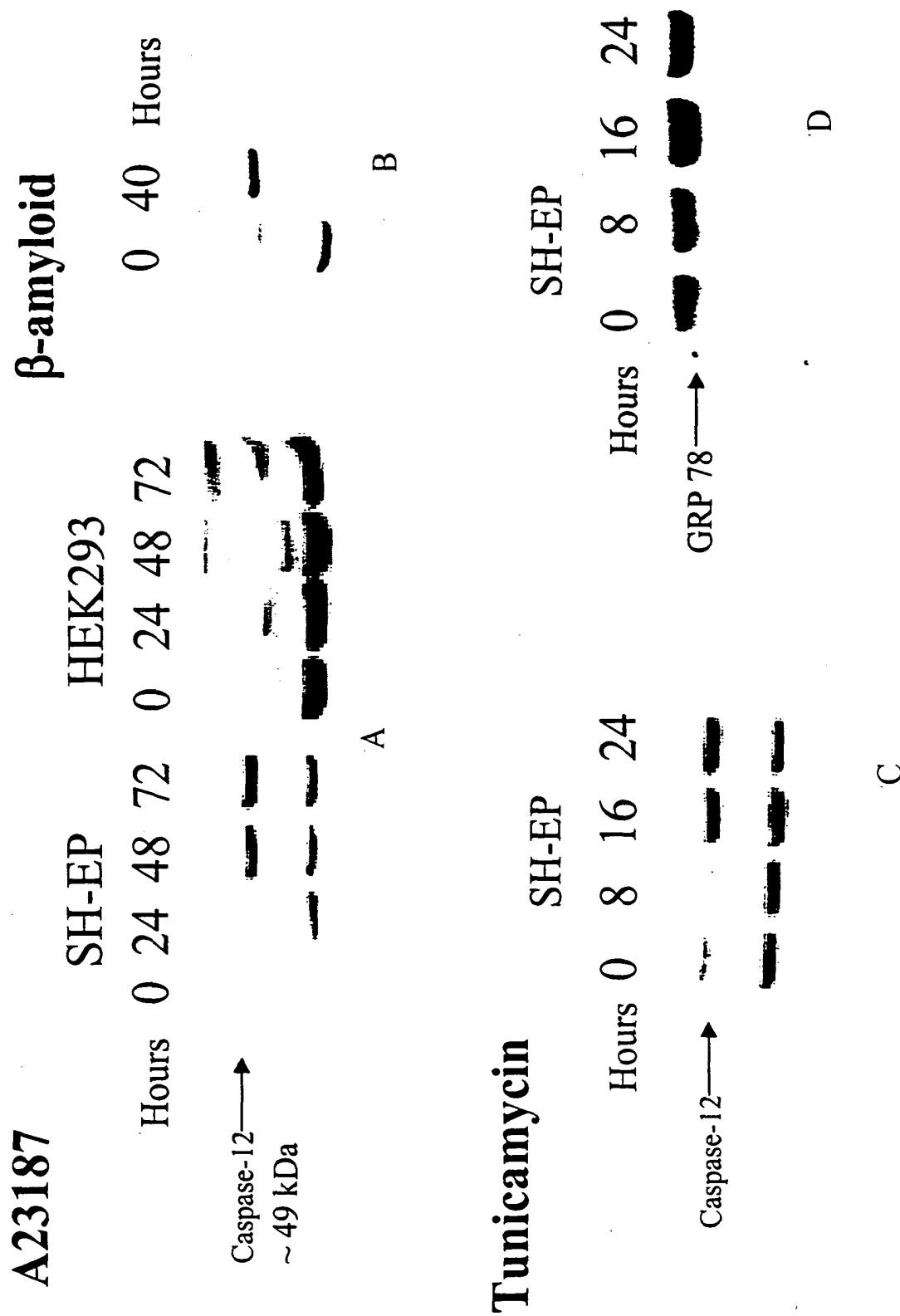


FIGURE 10

A.

Untreated

A23187

A23187, CHX

A23187, UV

A23187, α -Fas



Caspase-3 ↑

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FIGURE 11

SH-EP cells treated with A23187, UV +/- inhibitors

A.

1. 2. 3. 4.

Caspase-12



B.

Caspase-3



Calpain cleavage of recombinant caspase-12

